

AMENDMENT

Please enter the following amendments to the claims without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows:

In the Claims:

1. (Currently Amended) ~~Continuous~~ A method of continuous microencapsulation or multi-microencapsulation process of biologically active materials by means of in-situ *in situ* interfacial polymerization characterized in that the process is performed under continuous agitation [[and]] wherein the method comprises the following steps:

~~(a) in a first step a water phase is emulsified into an oil phase; wherein~~

forming a water in oil emulsion comprising a water phase, an oil phase, a polymerization initiator, an emulsifier and at least one biologically active ingredient(s), wherein:

[[a.1. a]] the polymerization initiator exists in the water phase,

[[a.2. an]] the emulsifier exists in the oil phase or in the water phase,

a.3. at least a the biologically active ingredient(s) exists in the oil phase and/or in the water phase;

~~(b) in a second step, a solution or dispersion in water that contains at least one hydrocolloid is added to the emulsion, wherein the hydrocolloid is polymerizable due to the polymerization initiator, this producing a phase inversion and the polymerization and crosslinking of the polymerizable hydrocolloid(s) onto the water in oil droplets~~ adding an aqueous solution or dispersion containing at least one hydrocolloid(s) which is polymerizable due to the polymerization initiator and thereby producing a phase inversion whereby there are drops of water in oil and polymerization and cross-linking of the hydrocolloid(s) onto the drops of water in oil;

~~(c) in a third step, a solution or dispersion in water that contains at least one protective colloid is added that begins to be deposited on the surface of the drops of water in oil, and to polymerize and cross-link with itself and the hydrocolloid, hydrocolloid~~ adding an aqueous solution or dispersion containing at least one protective colloid(s) and thereby depositing the protective colloid(s) on the surface of the drops of water in oil, whereby the protective colloid(s) polymerizes and cross-links with itself and the hydrocolloid(s);

~~(d) in a fourth step, a solution or dispersion in water of a surfactant is added to allow a reduction of the size of the water in oil drops;~~

~~(e) in a fifth step, during the process of reduction of size, the partially formed microcapsules are deagglomerated and reagglomerated, such that an enclosure of drops inside bigger drops eventually happens adding an aqueous solution or dispersion containing a surfactant and thereby reducing the size of the water in oil drops, whereby microcapsules or multi-microcapsules having a wall and suspended in water result; and~~

~~(f) when enough time has passed in order that the oil and/or water in oil drops are covered by at least one hydrocolloid and at least one protective colloid, the temperature is increased in order to strengthen the wall of the formed microcapsules or multi-microcapsules suspended in water subjecting the microcapsules or multi-microcapsules having a wall and suspended in water to a temperature increase to strengthen the wall.~~

2-4. (Canceled)

5. (Currently amended) ~~Process of microencapsulation~~ The method according to claim 1, ~~characterized in that wherein the hydrocolloid(s) of the second step and or the protective colloid(s) of the third step are chosen from the group: comprise a chitosan[[s]], a starch, a dextrin[[s]], a cyclodextrin[[s]], a cellulose[[s]], a pectin[[s]], an agar, an alginate[[s]], a carrageen[[s]], a gelatin[[s]], a seed gum[[s]], a xanthan gum, a guar gum, an acacia gum, an arabic gum, a Caraya gum, a Ceratonia siliqua gum, a Pysllium gum, a gelatin, a tragacanth[[s]], a lignin, a lignosulfonate[[s]], a saponine[[s]], a galactomanan[[s]], an arabanogalactam[[s]], a beta-glucan[[s]], an inulin; in all their isomeric and stereochemical forms, in all their variations regarding quantity and proportion of monomers or oligomers constituting the hydrocolloid, in their natural or derivatized forms, and as their salts of metal cations or nitrogenated, sulfurated or phosphorinated derivatives, an albumin, polyarboxylate[[s]] or a poliL-lactid.~~

6-13. (Canceled)

14. (Currently amended) ~~Process of microencapsulation of biologically active materials according to~~ The method of claim 1, ~~characterized in that wherein the aqueous solution [[of]] containing at least one hydrocolloid(s) contains comprises~~ a binary or ternary mixture of the hydrocolloids.

15-22. (Canceled)

23. (Currently amended) ~~Process of microencapsulation~~ The method according to claim 1, ~~characterized in that after the~~ wherein the method comprises drying ~~[[of]]~~ the microcapsules or multi-microcapsules, ~~these are~~ and then reformulating~~[[ed]]~~ and dispersing~~[[ed]]~~ the microcapsules or multi-microcapsules in an oil phase or in a gel or in a~~[[ny]]~~ semi-solid material or an ethanolic solution or another organic solvent.

24-39. (Canceled)

40. (Currently amended) ~~Process of microencapsulation of biologically active materials~~ The method according to claim 1, ~~characterized in that at least one of~~ wherein the biologically active materials present in the formulation consist in ingredients ~~comprise~~ a probiotic bacteria, optionally acid lactic bacteria and more preferably chosen among the group: *Lactobacillus casei*, *L. acidophilus*, *L. rhamnosus*, *L. paracasei*, *L. gasseri*, *L. fennentum*, *L. plantarum*, *L. salivarius*, *L. crispatus*, *L. bulgaricus*, *L. fennentum*, *L. reuteri*, *Bifidobacterium infantis*, *B. bifidum*, *Streptococcus tennophilus*, *S. bovis*, *Enterococcus durans*, *E. faecalis*, *E. Gallinarum*, *Escherichia coli*, *Propionibacterium freudenreichii*, or bacteria or fungi or yeasts genetically modified in that the beneficial genes characterizing the beneficial properties of probiotic bacteria have been inserted.

41-83. (Canceled)

84. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that it~~ wherein the method is carried out under reduced pressure.

85. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that it~~ wherein the method is carried out in the presence of an inert gas.

86. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that it~~ wherein the method is carried out protected from visible or ultraviolet light.

87. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that~~ wherein the continuous agitation is ~~the emulsions and reduction of particle size~~ are performed at an agitation speed of 3000 to 25000 rpm.

88. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that the size of the~~ wherein droplet~~[[s]]~~ size in ~~[[of]]~~ the water in oil emulsion ~~of the first step~~ is ~~[[of]]~~ 50-500 μm .

89. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 88 ~~characterized in that the size of wherein the droplet[[s]] size of the emulsion of the first step is 70-200 µm.~~

90. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein the hydrocolloid(s) of the second step and the protective colloid(s) of the third step are added together in the form of an aqueous solution or dispersion.~~

91. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein the protective colloid(s) belong to [[the]] a chemical group of hydrocolloids.~~

92. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein the oil phase is comprised of comprises an hydrogenated oil, [[or]] a wax, or a honey.~~

93. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein one of the emulsifier[[s]] used is based in comprises a soya containing compound[[s]].~~

94-95. (Canceled)

96. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein the emulsifier used in the fourth step has a HLB of 12-14.~~

97. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein the hydrocolloid(s) comprises a xanthan gum is added at any stage of the process wherein a hydrocolloid is used.~~

98. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein the hydrocolloids used in second step are of the type of hydrocolloid(s) comprises an alginate[[s]].~~

99. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein the protective colloid(s) [[is]] comprises an arabic gum.~~

100. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein it is added a further biologically active ingredient(s) in any step of the process, is added in the form of a solution, dispersion or emulsion.~~

101. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein~~ the water phase~~[[s]] contain~~ comprises at ~~[[the]]~~ most 40% of an alcohol of molecular weight up to 144 units of atomic mass.

102. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein~~ the oil phase ~~consists in~~ comprises a fish oil with omega-3 fatty acids, ~~[[or in]]~~ an arachidonic acid enriched oil, or ~~[[in]]~~ a conjugated linoleic acid~~[[s]]~~.

103. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein~~ the oil phase ~~consists in~~ comprises a vegetable oil extract of flax oil or Baraga spp.

104. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein~~ the hydrocolloids used for forming the wall, ~~allow the release of the content of the microcapsules~~ breaks down at a pH lower than 3.

105. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~characterized in that wherein~~ the oil phase ~~contains~~ comprises vitamin E or ascorbyl palmitate and ~~at least one the water phase contains~~ comprises ascorbic acid.

106. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 ~~wherein for its use in production of foodstuffs enriched with biologically active materials, characterized in that:~~

the method is performed at about 30-70 °C, with the temperature increase being
about 60-100°C;
the emulsifier is food grade;
after subjecting the microcapsules or multi-microcapsules to the temperature
increase, the method further comprises adding a food grade viscosity modifier;
and, the microcapsules or multi-microcapsule have an average size of about 1-30
µm.

~~(a) the process is kept at about 30-70 °C until the finalization of the polymerization and cross-linking reactions and then the temperature is raised to about 60-100 °C in order to cure the microcapsules;~~

~~(b) the final microcapsules have an average size of about 1-30 µm;~~

~~(c) after the curing step, it is added a food-grade viscosity modifier;~~

~~(d) during the process only food-grade emulsifiers are used.~~

107. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 characterized in that wherein it is added an additional step of the method further comprises microbiological stabilization by means of pasteurization, UHT, sterilization, ozonization, ultraviolet light or gamma rays irradiation or addition of antimicrobial chemical agents.

108. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 characterized in that wherein the method further comprises an additional drying step is made at the end of the process in order to obtain dried the microcapsules or multi-microcapsules in the to form [[of]] a powder.

109. (Currently amended) ~~Process of microencapsulation according to~~ The method of claim 1 characterized in that wherein at the end of the process, the resulting suspension of the microcapsules or multi-microcapsules having a wall and suspended in water [[is]] are lyophilized or spray dried.

110. (Canceled)

111. (New) The method of claim 1 wherein the biologically active ingredient(s) comprises: a Lactobacillus casei, a L. acidophilus, a L. rhamnosus, a L. paracasei, a L. gasseri, a L. fennentum, a L. plantarum, a L. salivarius, a L. crispatus, a L. bulgaricus, a L. fennentum, a L. reuteri, a Bifidobacterium infantis, a B. bifidum, a Streptococcus tennophilus, a S. bovis, a Enterococcus durans, a E. faecalis, a E. gallinarum, a Escherichia coli, or a Propionibacterium freudenreichii.

112. (New) The method of claim 1 wherein the biologically active ingredient(s) comprises a genetically modified bacteria, fungus or yeast.